Effect of Winery Yeast Lees on Touriga Nacional Red Wine Color and Tannin Evolution

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Abstract: Red wine aging on lees is a winemaking practice used to achieve more rounded and less astringent wines. In two different trials, external yeast lees were added to a red wine and their effects on wine color and tannin evolution during aging were studied. Results indicated that the addition of yeast lees did not affect color stabilization during the studied period. Color compounds and condensed tannins were rapidly adsorbed to the yeast lees at the beginning of the experiment. There was a retarding effect on proanthocyanidin polymerization reaction by the addition of yeast lees, leading to the maintenance of low and medium molecular weight tannins in solution. Two different interactions were observed: first, proanthocyanidin adsorption by the yeast lees, primarily ones with the highest polymerization degree, and second, the retarding of proanthocyanidin polymerization, likely by the mannoproteins released by yeast lees. The age of the yeast lees was a factor in mannoprotein release and its effect in wine.

Key words: red wine, yeast lees, color, proanthocyanidins, mannoproteins

Red wine polyphenols, namely anthocyanins and proanthocyanidins, are one of the most important and studied topics in enology. Anthocyanins are responsible for wine red color and exist at wine pH in colored forms, particularly when condensed with flavanol units and acetaldehyde in polymeric pigments (Somers 1971, Dallas et al. 1996). Proanthocyanidins or condensed tannins are related to wine astringency, bitterness, and color and are polymers resulting from the condensation between flavan-3-ol units. They are composed of procyanidins ((+)-catechin and (-)-epicatechin) and prodelphinidins ((-)-epigallocatechin), sometimes esterified by gallic acid (Haslam 1980, Ricardo da Silva et al. 1990, Fulcrand et al. 1999).

Red wine aging on lees is a common winery practice (Fornairon-Bonnefond et al. 2002), with the objective of improving wine mouthfeel characteristics through the production of a more rounded and less astringent wine. Yeast lees also enhance bacterial activity and growth during malolactic fermentation (Ribéreau-Gayon et al. 1998). In addition to the use of red wine fine lees, external yeast lees can be added to wine. The external yeast lees may consist of a commercial enological product or may be by-products of winery alcoholic fermentation, such as lees produced in the fermentation and aging of other wines.

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Wine fine lees are a reservoir of microorganisms, comprised mainly of alcoholic fermentation yeasts and some bacteria, and of other components such as tartaric salts and organic residues (Fornairon-Bonnefond et al. 2002). Their composition is variable and depends on the yeast autolysis process, including the characteristics of the medium, the particular strain of yeast, and the fermentation temperature (Feuillat and Charpentier 1982). Autolysis is the breakdown of the yeast cell components by endogenous enzymes and is characterized by the loss of cell organization, the degradation of cell macromolecules, and the release of the breakdown products into the extracellular environment (Hernawan and Fleet 1995). The main products of autolysis are amino acids, proteins, polysaccharides, lipids, and nucleic acids.

Mannoproteins are the principal products derived from yeast autolysis. These polysaccharides are released into the medium after the action of the β-(1→3) glucanases on cell wall glucans (Feuillat et al. 1989). Escot et al. (2001) verified that tannin structure is affected by the addition of mannoproteins, reducing wine astringency, and suggested that these polysaccharides retain anthocyanins and tannins, preventing their precipitation. Riou et al. (2002) studied the interaction between seed tannins and wine polysaccharides in a model solution and concluded that mannoproteins have a protective effect on tannin aggregation, although they did not prevent their initial aggregation. Poncet-Legrand et al. (2007) showed in a model wine that mannoproteins of low and medium molecular weight (51 and 62 kDa) were more effective, probably due to a steric hindrance mechanism, although all mannoprotein fractions prevented tannin aggregation and precipitation. On the other hand, Guadalupe et al. (2007) verified that commercial added mannoproteins did not protect polyphenols from precipitation nor was wine color protected, although they did contribute to improved mouthfeel and structure, resulting in a more rounded and sweet wine. Guadalupe and

Ayestarán (2008) have shown that during wine aging there is a precipitation of mannoprotein-tannin and mannoprotein-pigments aggregates, suggesting that mannoproteins do not act as protective colloids.

Yeast lees can also react directly with wine polyphenols, mainly by adsorption mechanisms (Salmon et al. 2002), and this kind of interference results in a decrease of the free polyphenols of wine. The choice of yeast strain used in alcoholic fermentation is a key to preserving wine color during vinification (Medina et al. 2005). Polar condensed tannins, with more epigallocatechin units, are preferentially adsorbed by yeast lees and there is no preferential adsorption between low and high tannin polymers (Mazauric and Salmon 2005). The anthocyanins that remain adsorbed in the lees have a dominant blue color (Morata et al. 2003, Mazauric and Salmon 2006), resulting in an enhancement of the yellow anthocyanins in the medium. Vasserot et al. (1997) verified that the adsorption of anthocyanins to yeast lees results from the establishment of hydrogen bonds and suggested that the adsorption capacity depended on the anthocyanin polarity, with polar anthocyanins being the more reactive. Conversely, Mazauric and Salmon (2006) verified that anthocyanin adsorption on yeast lees does not follow a simple mechanism of hydrogen bonding and that there is no relationship between anthocyanin polarity and adsorption capacity.

Some winemakers add yeast lees that are by-products of white winemaking to red wines during aging in order to achieve more rounded wines with improved mouthfeel, rather than adding commercial enological products. Given that wine yeast lees are a great source of mannoproteins and that this winery by-product is a possible source of economically produced mannoproteins, the aim of this work was to determine the effect of adding winery-derived yeast lees to a *Vitis vinifera* L. cv. Touriga Nacional red wine on its color and tannin evolution during aging, according to typical practices used at the Dão Sul winery. Two types of yeast lees were used and were from the alcoholic fermentation of a *Vitis vinifera* L. cv. Encruzado white wine: one from the end of the aging process in oak barrels and the other from the end of the alcoholic fermentation.

Materials and Methods

Chemicals. All chemicals were of analytical reagent grade. All solvents were of HPLC grade. (+)-Catechin, (-)-epicatechin, (-)-epicatechin-3-O-gallate, and (-)-epigallocatechin were purchased from Extrasynthese (Genay, France). Toluene-α-thiol (phenylmethanethiol) was purchased from Fluka (Buchs, Switzerland).

Wines. *Vitis vinifera* L. cv. Touriga Nacional grapes, a red variety, were harvested manually and vinified at Dão Sul winery (Dão Region, Portugal) in 2009. The grapes (9000 kg) were destemmed and crushed into a stainless-steel vessel and a preparation of commercial maceration enzymes (Vinozym Vintage FCE, Novozymes, Bagsvaerd, Denmark) was added. After 24 hr cold prefermentation maceration at 15°C, the must was inoculated with a commercial activated *Saccharomyces cerevisiae* preparation (Lalvin QD145, Lallemand, Montreal, Canada). The alcoholic fermentation occurred for 10 days at 20 to 25°C. After the beginning of the alcoholic fermentation, the must was punched down for 20 min every 3 hr and was submitted to a rack and return program for 30 min each day, until the end of alcoholic fermentation. After the end of alcoholic fermentation, the free-run wine was transferred to another stainless-steel vessel for malolactic fermentation. The wine was analyzed for ethanol content, pH, volatile acidity, titratable acidity (TA), and free and total SO_2 according to Organisation International de la Vigne et du Vin official methods (OIV 2006). The wine chemical parameters were the following: alcohol content 13.6% v/v, TA 7.8 g/L expressed in tartaric acid, volatile acidity 0.26 g/L expressed in acetic acid, pH 3.75, residual sugars 2.3 g/L, 15 mg/L free SO_2, and 32 mg/L total SO_2.

White wine yeast lees preparation and trials in red wines. Two different white wine-derived yeast lees were prepared for addition to the red wine. For the first trial, labeled BE wine, the yeast lees were prepared from winery lees from the production of a *Vitis vinifera* L. cv. Encruzado white wine. Grapes were harvested in 2009 in the Dão Sul winery. The free-run juice was cooled at 5°C, pectinolytic enzymes were added (Novoclair Speed, Novozymes), and settled for 24 hr. After decanting, the juice was inoculated with a commercial active dry yeast preparation (*Saccharomyces cerevisiae* var. *bayanus,* Enoferm QA23; Lallemand); fermentation was at 15 to 18°C for three weeks. At the end of the fermentation, the wine was submitted to *bâtonnage* by manual stirring with a stainless-steel stick for 30 seconds per French oak barrel, with an interval between two and three days for one month after the end of alcoholic fermentation. The lees were allowed to settle and then stirred once again 100 days after the end of alcoholic fermentation. Yeast lees were taken from the white wine at the time it was separated from lees for racking, six months after the end of alcoholic fermentation. The lees were then centrifuged at 3500 rpm for 10 min, and the resulting pellet was added to the red wine in the winery-determined proportion of 5% (w/v) of the total volume.

In order to determine whether the age of the lees would influence color and tannin stabilization, a second trial, labeled LN wine, used yeast lees prepared from a newly fermented Encruzado clarified juice as described above. Four liters of Encruzado juice was fermented with the same commercial active dry yeast preparation as the white wine obtained for the BE trial. The fermentation occurred at controlled temperature (35ºC) for four days. This high temperature was used to accelerate the fermentation process. One day after the end of alcoholic fermentation the yeast lees were centrifuged at 3500 rpm for 10 min. The resulting pellet was added to the wine in the proportion of 0.8% (w/v), the same proportion that yeast lees represented in the white fermented wine.

The control, labeled T wine, consisted of wine with no addition of yeast lees. The three wines were kept in 2.5 L amber flasks at 35°C for 60 days. Samples of each wine were analyzed for color characterization and proanthocyanidin composition according to their degree of polymerization at 0, 6, 13, 19, 26, 47, and 60 days. The analyses were performed

in duplicate; if the duplicated values were quite different, then the analysis was repeated.

Color characterization. Wine color compounds were characterized using the spectrophotometric method described by Somers and Evans (1977). The wines were centrifuged for 10 min at 3.500 rpm, and absorbances were measured using a UV-Vis UV4 spectrophotometer (Unicam, Cambridge, UK).

Proanthocyanidin isolation. Wine proanthocyanidins were isolated according to published methods (Sun et al. 1999, Labarbe et al. 1999). Four milliliters of wine were injected onto a Toyopearl TSK HW-40F (Tosoh Corp., Tokyo, Japan) packed column (100 x 10 mm) and first washed with a solution of ethanol:water:TFA (55:45:0.05 v/v/v) to remove small molecules and monomeric flavanols. The wine oligomeric and polymeric proanthocyanidins were eluted with a solution of acetone:water (40:60 v/v). After evaporation of the tannin fraction at 30°C under vacuum, the proanthocyanidins were resuspended in 1 mL methanol for use in further analysis.

Proanthocyanidin fractionation. The proanthocyanidins were separated according to their degree of polymerization as described by Labarbe et al. (1999). One milliliter of proanthocyanidin methanol solution was precipitated by chloroform on the top of a glass powder column (50 x 10 mm). The elution gradient (chloroform/methanol) was applied as: FI, 75:25 (v/v); FII, 70:30 (v/v); FIII, 65:35 (v/v); FIV, 60:40 (v/v); FV, 55:45 (v/v); FVI, 50:50 (v/v); FVII, 45:55 (v/v); FVIII, 0:100 (v/v). After evaporation of each tannin fraction at 30°C under vacuum, the proanthocyanidins were resuspended in 0.5 mL methanol. The eight fractions were analyzed by HPLC after thiolysis to determine their structural characteristics.

Proanthocyanidin characterization. The proanthocyanidins were submitted to depolymerization in the presence of toluene-α-thiol in an acidic medium as described by Monagas et al. (2003). One hundred microliters of toluene-α-thiol 5% in methanol 0.2 M HCl were added to 100 µL proanthocyanidin solution in a hermetically sealed glass tube. The mixture was heated to 55°C on a water bath for 7 min. Ten microliters of the prepared solution were immediately injected in a HPLC system equipped with a Lachrom L-7100 Pump (Merck Hitachi, Tokyo, Japan), a Gemini C18 110 A 150 x 3.0 mm column (Phenomenex, Torrance, CA), and a UV/Vis 2487 Dual Wavelength detector (Waters, Milford, MA), in the following elution conditions: 0.8 mL/min flow with a linear gradient of 15 to 75% of a solution of acetonitrile/water/formic acid (80:18:2, v/v/v) in a solution of water/formic acid (98:2, v/v), in an 18 min run. The detection was monitored at 280 nm and 320 nm. The amounts of monomers and of toluene-α-thiol adducts released were calculated from the areas of the chromatographic peaks at 280 nm by comparison with calibration curves (Rigaud et al. 1991).

Polysaccharide characterization. In order to characterize the polysaccharide composition of the white wine-derived lees, lees from both trials were digested with a commercial preparation of β-glucanases from *Trichoderma harzanium* as described elsewhere (Moine-Ledoux et al. 1997). The schematic procedure for the characterization of the yeast lees is shown in Figure 1. The yeast lees were diluted in distillated water in a concentration of 15% (v/v). Glucanex (Novozymes) was added in a concentration of 1% of the lees weight and the mixture was kept at 42°C for 5 hr. After this period the mixture was centrifuged, dialyzed against water, and freeze-dried. The mixture was dissolved in water and injected onto a Concanavalin-A Sepharose 4B (GE Healthcare Bio-Sciences, Uppsala, Sweden) packed column (100 x 10 mm), and eluted with a sodium acetate-HCL 50 mM pH 5.6, NaCl 150 mM, $CaCl_2$ 1 mM, $MgCl_2$ 1 mM, and $MnCl_2$ 1 mM buffer solution at 0.8 mL/min, monitored by a refraction index and a 254 nm wavelength detector as described by Gonçalves et al. (2002), which constituted the nonretained fraction (FNR). The retained fraction (FR) was eluted with the same buffer solution with methyl α-D-mannopyranoside added to 500 mM. Both the nonretained and retained fractions were dialyzed against water for seven days, at 4°C. The process was monitored with a conductivity meter (model 220; Denver Instrument, Bohemia, NY). After freeze drying, each fraction (1 g/L) was injected onto a FPLC system equipped with a Pharmacia LKB Pump P-50 and a 12HR 10/30 FPLC size-exclusion column (GE Healthcare Bio-Sciences) and eluted with an ammonium acetate 0.3 M buffer solution at 0.3 mL/min and monitored by a refractive index detector (model LC-30 RI; Perkin-Elmer, Waltham, MA) modified with a light-emitting diode (Cromolab, Queijas, Portugal) and a WellChrom Spectro-Photometer K-2501 wavelength detector (Knauer, Berlin, Germany) at 254 nm. Calibration of the system was performed with Shodex P-82 Pullulan standards (Showa Denko, Kanagawa, Japan).

Carbohydrate composition was determined by gas chromatography after derivatization of the samples into their alditol acetates according to Albersheim et al. (1967). 100 µL *myo*-inositol solution (1 mg/mL) and 1 mL trifluoroacetic acid 2 M were added to 1 mL polysaccharide solution (1 mg/mL). After hydrolysis at 120ºC, for 75 min, the mixture was washed with 5 mL water and dried. 500 µL of a saturated sodium borohydride solution in ammonia was added and the mixture reacted for 2 hr at room temperature. The reaction was stopped by adding some drops of glacial acetic acid and the mixture was washed with 5 mL of a solution of 1% HCl in methanol and dried. 150 µL pyridine and 150 µL acetic anhydride were added to the mixture and it reacted for 12 hr at room temperature. The reaction was stopped by adding a drop of water in an ice bath. The mixture was washed with 5 mL water, followed by 1 mL ethanol, and dried. The alditol acetates were extracted to 200 µL chloroform and quantified on a CE Instruments GC 8000 Top gas chromatograph (Thermo Fisher Scientific, Milan, Italy) with a capillary column Zebron ZB-Wax 10 60 x 0.25 mm, 0.25 µm film (Phenomenex) and a FID detector. The column temperature was initially set at 220°C for 4 min and raised to 235°C at 10°C/min, maintaining this temperature for 5 min. Hydrogen was used as carrier gas at 1 mL/min. *myo*-Inositol was used as the internal standard, and sugar quantification was made after determination of each sugar response factor using pure sugars.

The total protein content was determined as described by Lowry et al. (1951) using bovine serum albumin fraction

V (Sigma-Aldrich, St. Louis, MO) for the calibration curve. Total polysaccharide content was determined by the phenol-sulfuric method as described by Dubois et al. (1956) using glucose (Panreac, Barcelona, Spain) for the calibration curve, as glucose was the most common sugar for calibration curve referred to in the literature.

Sensory analysis. The three wines (T, BE, and LN) were submitted to a tasting panel of 11 experts from the Technical University of Lisbon and the Dão Sul wine company. All tasters had previous training in sensory analysis. Wines were tasted at the end of the experiment at 18°C, in an acclimatized tasting room at 20°C. The wines were tasted one time by each taster in one single session, using official tulip-shaped glasses with 60 mL wine. A descriptive tasting sheet was used with the following parameters: color intensity; aroma: fruity, floral, intensity, persistency, equilibrium; mouthfeel: body, bitterness, astringency, acidity, persistency, equilibrium; and overall balance. These parameters were generated according to the objective of the experiment. All parameters were classified on a scale of 1 (absent) to 5 (very intense), except for aroma equilibrium, mouthfeel equilibrium, and overall balance, for which the classification was on a scale of 1 (bad) to 5 (excellent). All attributes were classified according to their perceived intensity by the tasters. Significant differences between results were analyzed using Statistica 6.0 software (Statsoft, Tulsa, OK). The values for each tasting descriptor were submitted to a one-way analysis of variance (ANOVA) as the tasting panel was trained. Differences between samples always refer to significant differences with at least $p < 0.05$.

Results

Characterization of white wine yeast lees. The results obtained in the characterization of white wine yeast lees are represented in Table 1. The sugar composition of the mannoproteins is presented as percentages of each fraction (peak) from the FPLC chromatography. The main goal of this characterization was to determine whether the BE and LN lees were chemically different, even though the grape must and the yeast strain used in the alcoholic fermentation were the same for both lees. The total colloids extracted from the yeast lees by the action of β-glucanase represented 1.2% and 1.3% (w/w) of the centrifuged yeast lees total weight for BE and LN, respectively. In both cases, the concanavalin-A nonretained fractions represented the lesser amount of the total colloids obtained from the digestion of the yeast lees (21%

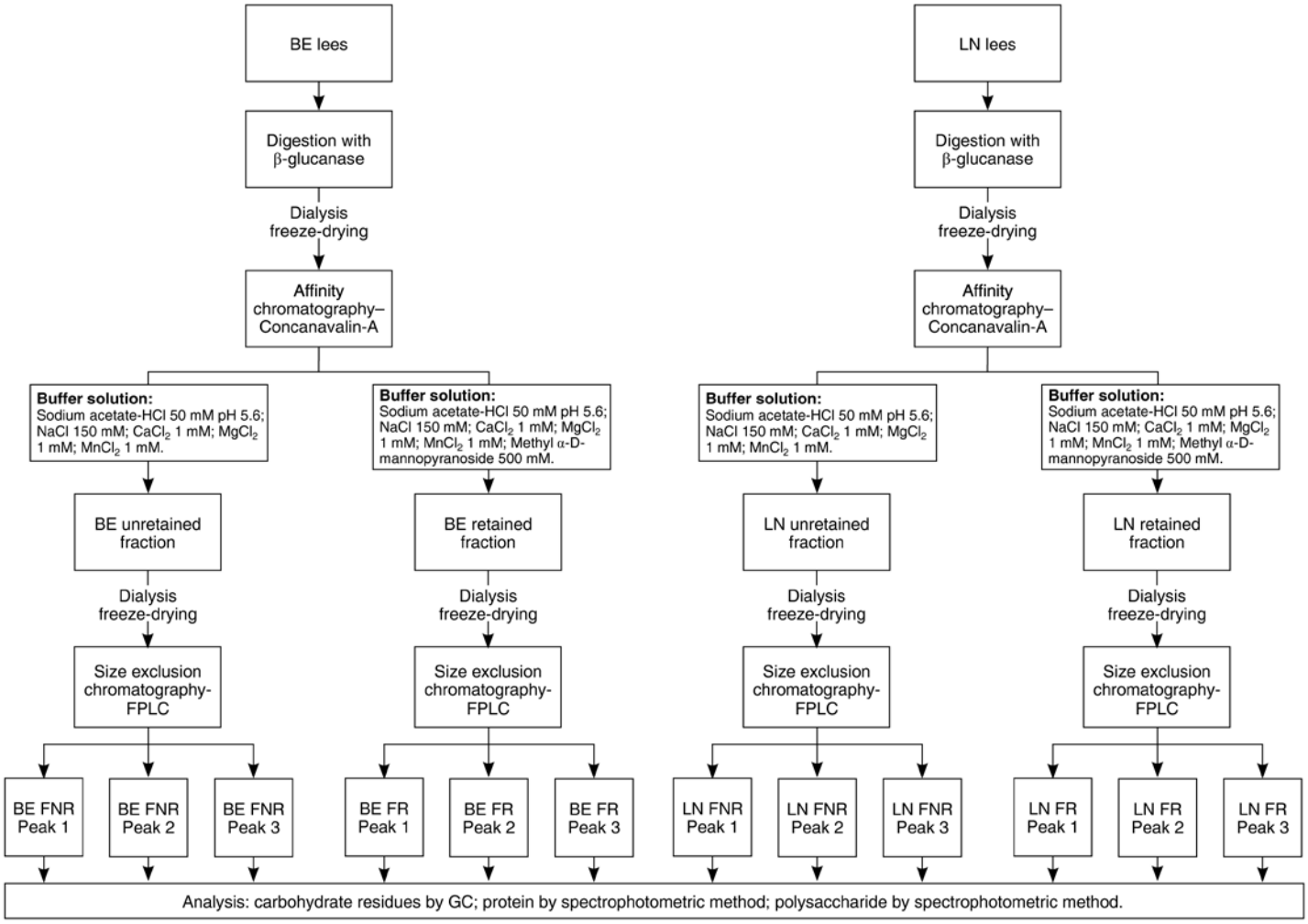

Figure 1 Schematic representation of the yeast lees characterization procedure of winery by-product older lees (BE) and newly fermented lees (LN); concanavalin-A column nonretained fraction (FNR) and retained fraction (FR).

for BE and 13% for LN). The retained fraction, where the mannoproteins should be, represented 56% and 65% of the BE and LN total colloids, respectively.

The molecular weight (MW) separation of the concanavalin-A nonretained (BE FNR) and retained (BE FR) fractions was determined (Figure 2A, B). For BE FNR, there were three different peaks with an average molecular weight of 177, 23, and 3 kDa, respectively. These peaks presented high values of arabinose and galactose, with the 177 kDa and 23 kDa peaks presenting ~9% and 90% and the 3 kDa peak presenting 18% and ~55% of arabinose and galactose, respectively. For the residual sugars and protein composition of each molecular weight separated fraction, the three different peaks were possibly polysaccharides rich in arabinose and galactose (PRAGs). As the yeast lees were obtained from the fermentation of a white wine must, they may have contained grape-derived polysaccharides, similar to PRAGs.

The BE FR sample showed two well-defined peaks with average molecular weights of 151 and 23 kDa and an intermediate zone that seemed to be a third compound with an average molecular weight of 54 kDa. The 151 kDa peak had a similar content of mannose (52%) and glucose (47%) and a proteic part (Table 1). As the added yeast lees were not the pure fermented yeasts, the polysaccharide found could be from an incomplete hydrolysis of this polysaccharide (glucan-mannoprotein polymer) or its origin is other than the yeasts. The two other peaks, 54 and 23 kDa MW, had higher mannose content (95% and 96%, respectively) and also a proteic part; given this composition, they represented mannoproteins.

The molecular weight distributions of the concanavalin-A retained (LN FR) and nonretained (LN FNR) fractions indicate three different macromolecules in both samples (Figure 2C, D). The LN FNR average molecular weights were 159, 23, and 2 kDa (Table 1). The first peak (159 kDa MW) had high arabinose (15%) and galactose (84%) content and was possibly a PRAG. The second peak (23 kDa MW) had very high protein content and was possibly a glycoprotein. The third peak (2 kDA MW) had very high arabinose (40%) and galactose (60%) content and could be a PRAG of low molecular weight.

The LN FR average molecular weights were 154, 50, and 25 kDa. The first peak (154 kDa MW) had high protein content, with glucose the main sugar (60%) and was possibly a glycoprotein. The second peak (50 kDa MW) had high mannose (89%) content and a proteic part, thus indicating a mannoprotein. The third peak (25 kDa MW) also had mannose (67%) as the main sugar and a proteic part, again indicating a mannoprotein.

Mannoprotein content in the newly fermented yeast lees (LN) was higher than in the older fermented yeast lees (BE), likely because BE was in contact with the wine for a longer period. Thus, a greater amount of mannoproteins was released into the medium, which resulted in a decrease in mannoprotein content in the lees when they were added to the red wine.

Effect of yeast lees addition on TPI and color compounds. The evolution of color parameters is shown in Figure 3. The total polyphenol index (TPI) (I_{280}) showed variations over time for the T wine sample and it evolved for a final higher value than the BE and LN wine samples. The BE wine slightly decreased in the first day of the experiment,

Table 1 Characterization of two samples of white wine yeast lees: older fermented wine yeast lees from a six-month wood aging process (BE) and newly fermented yeast lees (LN). Characterization of the concanavalin-A nonretained (FNR) and retained (FR) fractions for molecular weight (MW), polysaccharides (polysac. %), proteins (%), and residual sugars (%).

Sample	MW (kDa)[c]	Polysac. (%)[c]	Proteins (%)[c]	Sugars (%)[c,d]						
				Fuc	Rha	Ara	Xyl	Man	Glc	Gal
BE wines (1.2%)[a]										
FNR (21%)[b]										
Peak 1	177 ± 17	71.1 ± 0.2	28.9 ± 0.2	n.d.	n.d.	9.0 ± 0.0	n.d.	n.d.	n.d.	91.0 ± 0.0
Peak 2	23 ± 1	85.5 ± 0.3	14.5 ± 0.3	0.7 ± 0.9	1.8 ± 2.5	9.2 ± 2.0	1.4 ± 2.0	n.d.	n.d.	86.9 ± 1.5
Peak 3	3 ± 0	67.0 ± 0.0	32.2 ± 0.0	2.7 ± 0.0	8.9 ± 0.0	18.3 ± 0.0	15.1 ± 0.0	n.d.	n.d.	54.9 ± 0.0
FR (56%)[b]										
Peak 1	151 ± 11	85.2 ± 0.3	14.8 ± 0.3	n.d.	n.d.	0.1 ± 0.1	0.4 ± 0.2	52.2 ± 6.4	47.1 ± 6.4	0.2 ± 0.3
Peak 2	54 ± 4	92.7 ± 0.3	7.3 ± 0.3	n.d.	n.d.	0.4 ± 0.2	0.1 ± 0.1	95.3 ± 0.9	3.8 ± 1.3	0.5 ± 0.1
Peak 3	23 ± 1	94.0 ± 0.5	6.0 ± 0.5	n.d.	n.d.	0.4 ± 0.0	n.d.	95.9 ± 0.3	3.7 ± 0.3	n.d.
LN wines (1.3%)[a]										
FNR (13%)[b]										
Peak 1	159 ± 2	80.1 ± 2.3	19.9 ± 2.3	0.9 ± 1.2	0.0 ± 0.0	14.5 ± 4.2	0.4 ± 0.5	n.d.	n.d.	84.3 ± 2.4
Peak 2	23 ± 1	21.3 ± 1.8	78.7 ± 1.8	3.3 ± 0.0	8.1 ± 3.0	23.1 ± 0.0	n.d.	n.d.	n.d.	63.3 ± 0.0
Peak 3	2 ± 0	46.3 ± 3.5	53.7 ± 3.5	n.d.	n.d.	40.5 ± 0.0	n.d.	n.d.	n.d.	59.5 ± 0.0
FR (65%)[b]										
Peak 1	154 ± 11	25.9 ± 2.6	74.1 ± 2.6	n.d.	0.2 ± 0.0	0.2 ± 0.0	n.d.	32.9 ± 0.3	60.4 ± 1.3	6.5 ± 2.0
Peak 2	50 ± 3	81.8 ± 0.3	18.2 ± 0.3	n.d.	0.1 ± 0.1	0.1 ± 0.0	n.d.	88.6 ± 5.8	8.9 ± 4.3	2.2 ± 1.5
Peak 3	25 ± 1	92.6 ± 0.3	7.4 ± 0.3	n.d.	n.d.	0.4 ± 0.1	0.1 ± 0.0	67.1 ± 7.0	29.5 ± 6.7	2.8 ± 0.4

[a]Total colloids (%), extracted from yeast lees after digestion with β-glucanase in aqueous medium.
[b]Distribution in total colloids (%) after concanavalin-A affinity chromatography.
[c]After FPLC size-exclusion chromatography.
[d]Fuc: fucose; Rha: rhamnose; Ara: arabinose; Xyl: xylose; Man: mannose; Glc: glucose; Gal: galactose: n.d.: not detected.

increasing until day 19, and then stabilized. The LN wine maintained the same total polyphenol index until the end of the experiment. The color intensity (CI) of the T wine maintained a constant evolution with no variations until the end of the experiment, when it increased slightly. Both the BE and LN wines had an initial rapid decrease in color intensity due to yeast lees addition. All hue modalities showed a similar behavior, increasing through time and with similar values. The BE wine had a slightly higher final value, followed by T, and then LN.

Polymeric pigments (PP) represent longer chain colored compounds resulting from condensation reactions between anthocyanins and flavanol units (Somers 1971). Their evolution was similar to color intensity, total anthocyanin content (TA), and total pigments (TP).

Despite the results from the planned trial, it was observed two years later that the LN wine had a color intensity slightly higher (4.5) than the T control wine (4.3) and the BE wine (3.9). It was also observed at long term a lower degree of polymerization of color pigments in LN wine (53%) compared with T control (59%) and BE (57%) wines.

Effect of yeast lees on proanthocyanidins. The T wine, with no yeast lees added, showed a continual decrease in total proanthocyanidins from the beginning to the end of the experiment (Figure 4A). In comparison, both wines with yeast lees added (BE and LN) had an initial greater decrease in proanthocyanidin content, observable from day 0 to day 5: in BE wine it decreased ~690 mg/L and in LN wine it decreased ~521 mg/L. Between day 5 and the end of the experiment (day 60), BE wine had constant proanthocyanidin content with almost no variation. In LN wine, proanthocyanidin content decreased ~136 mg/L from day 5 until day 60. In comparison, T wine had a decrease of 270 mg/L from day 5 until the end of the experiment, although it finished with higher proanthocyanidin content.

The evolution of the mean degree of polymerization (mDP) of the wine with no added yeast lees (T wine) showed a slow decrease through time, starting with 15.2 mDP and ending with 11.1 mDP (Figure 4B). Wine with older fermented lees (BE) had a more rapid decrease in the beginning of the experiment from day 0 to day 5, after which it stabilized until the end (day 60) (9.1 mDP). Wine with newly fermented yeast lees (LN) had a similar evolution to T wine, finishing with the highest mDP (12.5).

The amount of proanthocyanidins per mDP interval and by experiment was determined (Figure 5). In each day of sampling, the wines were fractioned regarding proanthocyanidins and the respective mean degree of polymerization. The mDP values for each of the eight purified fractions varied between 3.6 and 33.0 (data not shown), resulting in a wide range of values. The results for the eight tannin fractions obtained on the glass powder separation column were grouped in intervals of mDP values, from 3 to 8, 8 to 14, 14 to 20, and 20 to 33 to facilitate evaluation of the results.

For the T control wine, on day 5 the proanthocyanidin distribution was similar to day 0 for all mDP intervals, but on the day 26, there was an increase in the percentage of proanthocyanidins in the interval 8–14 and a decrease in the

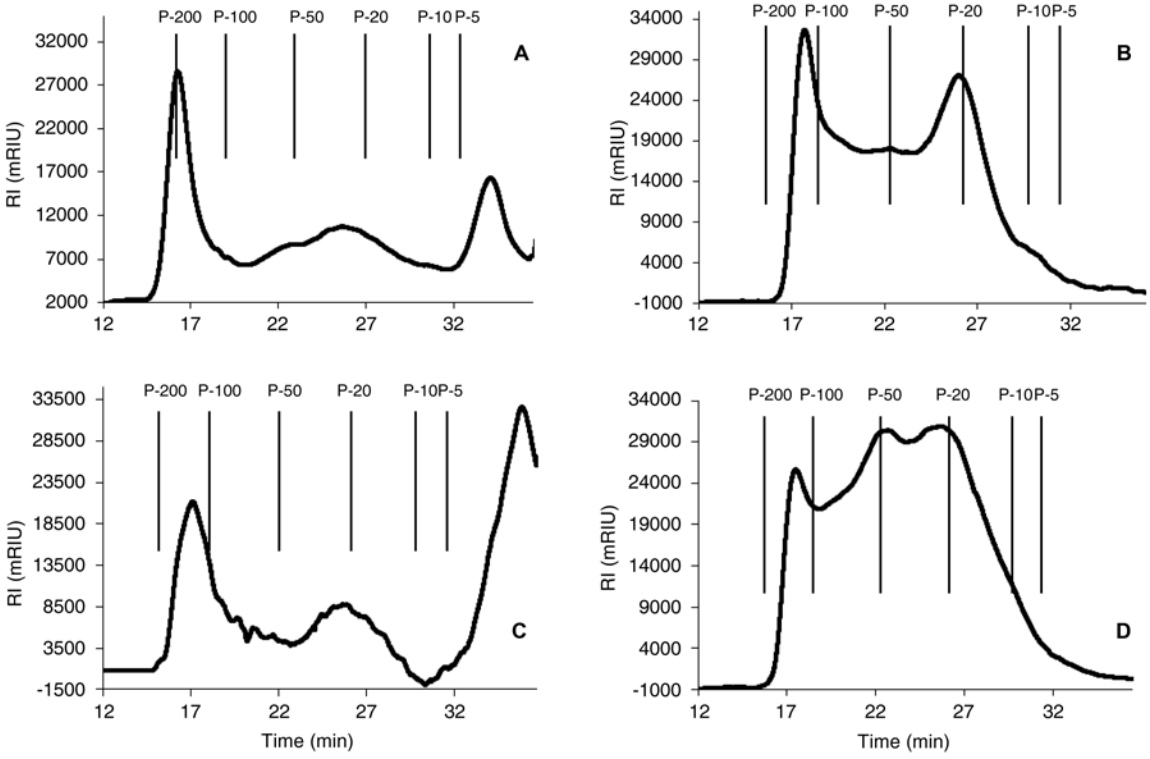

Figure 2 Molecular weight distribution of the concanavalin-A column nonretained (FNR) and retained (FR) fractions of the external yeast lees polysaccharides by FPLC, with the elution of the pullulan standards: (**A**) winery by-product older lees nonretained fraction (BE FNR); (**B**) winery by-product older lees retained fraction (BE FR); (**C**) newly fermented lees nonretained fraction (LN FNR); and (**D**) newly fermented lees retained fraction (LN FR).

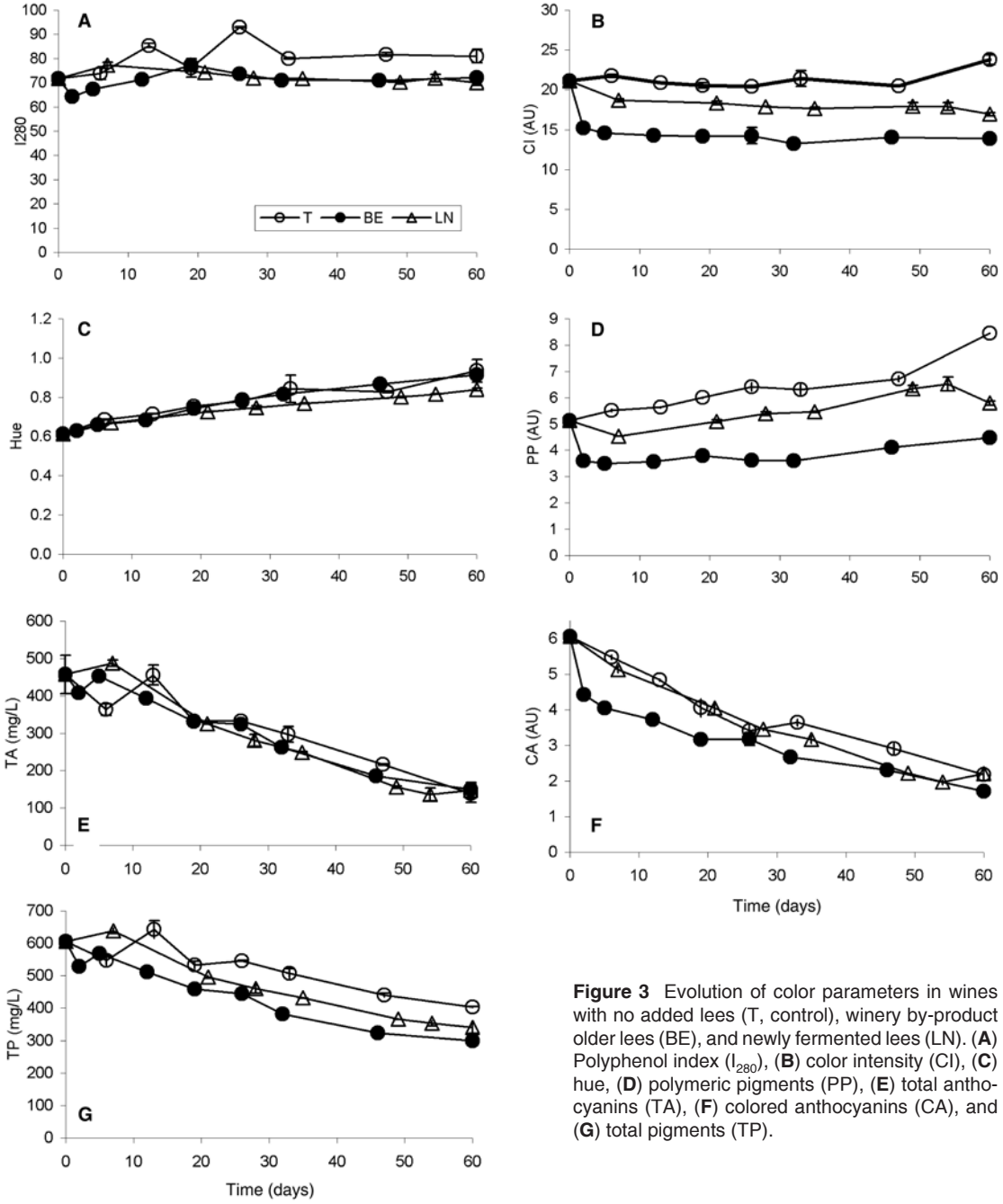

Figure 3 Evolution of color parameters in wines with no added lees (T, control), winery by-product older lees (BE), and newly fermented lees (LN). (**A**) Polyphenol index (I_{280}), (**B**) color intensity (CI), (**C**) hue, (**D**) polymeric pigments (PP), (**E**) total anthocyanins (TA), (**F**) colored anthocyanins (CA), and (**G**) total pigments (TP).

interval 20–33. On the final day of the experiment, the presence of proanthocyanidins with an mDP ranging 20–33 was no longer observed. In the BE wine, the interval 20–33 disappeared earlier, since this fraction was no longer present on the day 26. It was possible to observe mainly the presence of proanthocyanidins belonging to the intervals 3–8 and 8–14. Together they represented 92% of the total tannins. Wines with LN yeast lees retained proanthocyanidins mainly belonging to the interval 14–20, which represented 54% of total tannins.

The evolution of wine proanthocyanidin galloylation and prodelphinidin percentage is shown (Table 2). For the T control wine, there were no differences between the several sampling dates both for galloylation and prodelphinidin percentage. Wine with newly fermented yeast lees added (LN) had the same evolution as T wine. Wine with older fermented yeast lees added (BE) had a decrease in both galloylation and prodelphinidin percentage between day 0 and day 60. Proanthocyanidins belonging to the 3–8 mDP interval decreased 0.7% in galloylation between day 0 and day 60 and proanthocyanidins in the 8–14 mDP interval decreased 0.9% in the same period. Regarding the evolution of prodelphinidin percentage from the beginning to the end of the experiment, proanthocyanidins in the 8–14 mDP interval decreased 4.3% and proanthocyanidins in the 14–20 mDP interval decreased 4.9%.

Sensory analysis. In terms of color intensity and aroma attributes, sensory analysis revealed no significant ($p < 0.05$) differences among the three wines (Table 3). For mouthfeel,

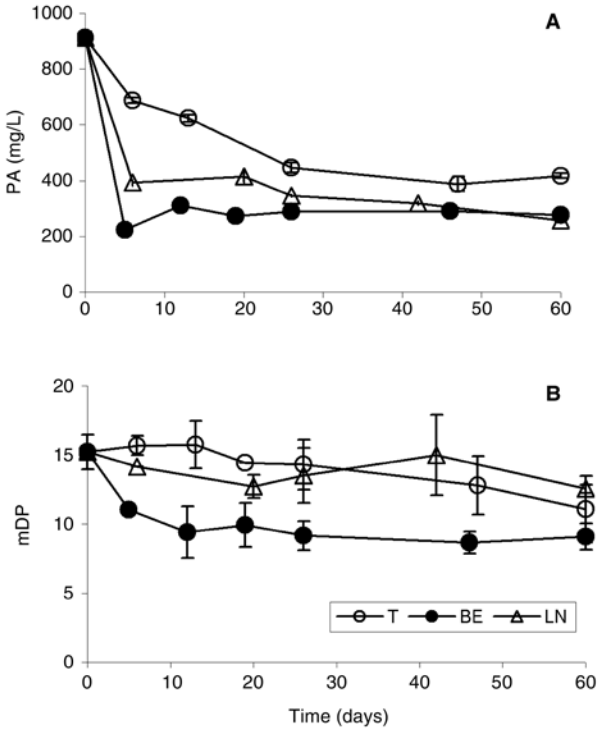

Figure 4 Evolution of (**A**) total proanthocyanidins (PA) and (**B**) overall mean degree of polymerization (mDP) in wines with no added lees (T, control), winery by-product older lees (BE), and newly fermented lees (LN).

there were some significant differences among the wines. Wine body was significantly lower ($p < 0.05$) in BE (2.64) wine than in T (3.27) and LN (3.45) wines, with no significant difference ($p < 0.05$) between the last two. The BE wine had a significant lower ($p < 0.05$) persistence than the T control wine and a lower ($p < 0.05$) mouthfeel equilibrium than the LN wine. Although there were no significant differences ($p < 0.05$) among the three wines for the overall balance, the LN wine (yeast lees added) was rated the highest (3.00) by the tasting panel, followed by the T control wine (2.91), and then the BE wine (2.36).

Discussion

Yeast lees addition and TPI and color compounds. The T wine had a higher total polyphenol index (I_{280}) than the other two wines, likely due to some initial adsorption of polyphenols to lees. This adsorption was more evident in the BE wine, possibly because the amount of yeast lees added to the LN wine (0.8% [w/v]) was much less than that added to BE wine (5% [w/v]). Guadalupe et al. (2007) found that wines with added external mannoproteins had a greater decrease in the total polyphenol index due to a possible precipitation of polyphenol-mannoproteins aggregates. Salmon et al. (2002) verified that yeast lees were highly reactive with wine polyphenols when the lees were cultivated in the absence of polyphenols. As the yeast lees used on this experiment were produced in a white wine fermentation, the polyphenols interacted immediately with the added yeast lees, resulting in a decrease of wine polyphenol content.

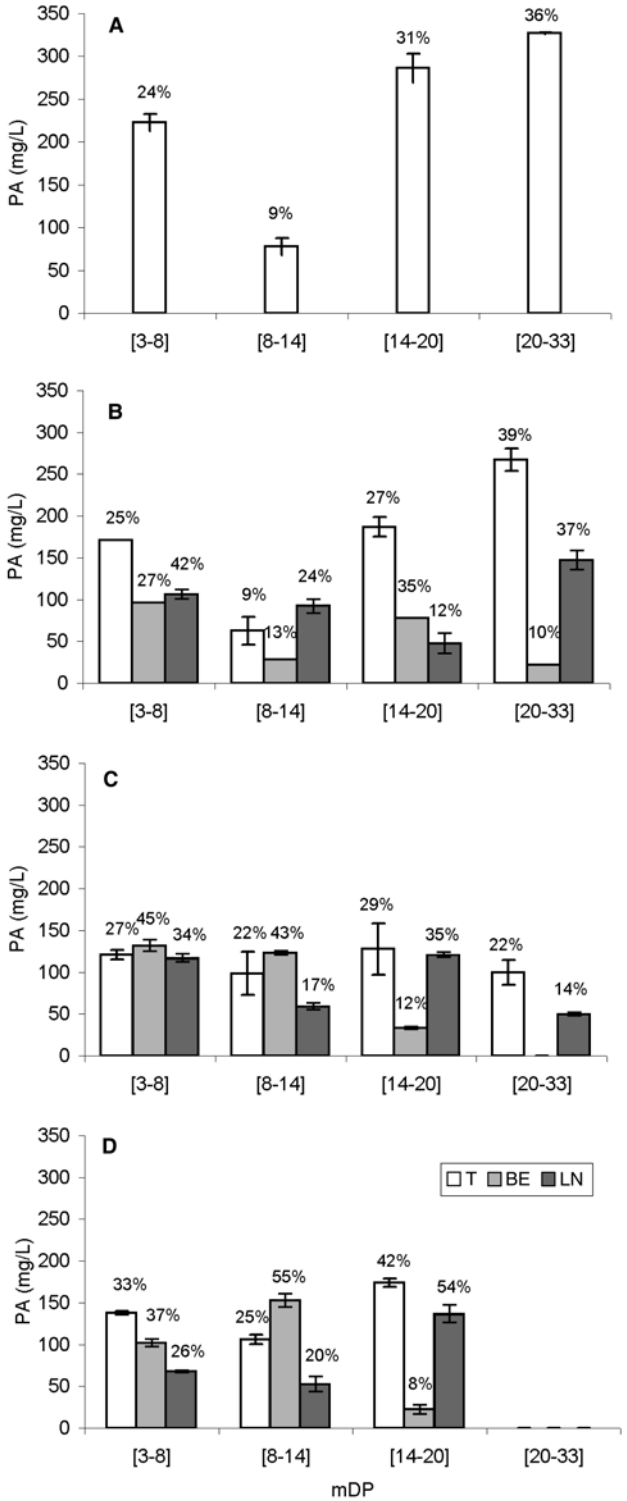

Figure 5 Evolution of the amount of proanthocyanidins per mDP interval and in wines with no added lees (T, control), winery by-product older lees (BE), and newly fermented lees (LN): (**A**) day 0, (**B**) day 5, (**C**) day 26, and (**D**) day 60. Relative percentages of proanthocyanidins per mDP interval for each sample are also shown.

Color intensity had an initial rapid decrease at the beginning of the experiment for BE and LN wines. Color intensity is determined by colored anthocyanins and polymeric pigment content. Vasserot et al. (1997) verified that the amount of adsorbed anthocyanins linearly increases as a function of

Table 2 Structural characteristics of wines in terms of percentages of galloylation (%Gal) and prodelphinidins (%Prodel) of wine proanthocyanidins, in different mean degree of polymerization intervals (mean ± SD).

Sample[a]/ days	Total proanthocyanidins		3-8 interval		8-14 interval		14-20 interval		20-33 interval	
	%Gal	%Prodel	%Gal	%Prodel	%Gal	%Prodel	%Gal	%Prodel	%Gal	%Prodel
T wine										
0	2.5 ± 0.3	24.0 ± 1.0	2.4 ± 0.2	18.7 ± 0.6	2.9 ± 0.3	24.1 ± 0.1	2.5 ± 0.2	25.7 ± 0.4	2.6 ± 0.3	26.3 ± 1.8
5	2.9 ± 0.0	27.4 ± 0.6	2.5 ± 0.0	19.8 ± 0.0	2.8 ± 0.0	25.0 ± 0.0	2.9 ± 0.0	26.6 ± 0.6	3.0 ± 0.1	32.4 ± 1.0
26	2.3 ± 0.4	25.5 ± 3.6	2.2 ± 0.8	21.0 ± 5.0	2.4 ± 0.2	24.9 ± 3.3	2.3 ± 0.3	27.9 ± 2.2	2.0 ± 0.4	27.7 ± 4.4
60	2.8 ± 0.3	25.6 ± 2.3	2.5 ± 0.7	21.6 ± 4.7	2.8 ± 0.2	25.3 ± 1.5	2.9 ± 0.1	28.5 ± 1.0	-	-
BE wine										
0	2.5 ± 0.3	24.1 ± 1.0	2.4 ± 0.2	18.7 ± 0.6	2.9 ± 0.3	24.1 ± 0.1	2.5 ± 0.2	25.7 ± 0.4	2.6 ± 0.3	26.3 ± 1.8
5	2.3 ± 0.0	24.2 ± 4.5	2.1 ± 0.0	18.9 ± 1.3	2.1 ± 0.0	22.0 ± 0.0	2.0 ± 0.0	27.9 ± 0.0	2.0 ± 0.0	24.9 ± 0.0
26	1.9 ± 0.2	18.0 ± 0.6	1.8 ± 0.3	15.8 ± 0.3	1.8 ± 0.1	19.9 ± 0.9	2.4 ± 0.3	18.8 ± 0.9	-	-
60	1.9 ± 0.1	18.4 ± 1.3	1.7 ± 0.1	15.8 ± 2.5	2.0 ± 0.1	19.8 ± 0.5	2.1 ± 0.2	20.8 ± 1.0	-	-
LN wine										
0	2.5 ± 0.3	24.1 ± 1.0	2.4 ± 0.2	18.7 ± 0.6	2.9 ± 0.3	24.1 ± 0.1	2.6 ± 0.2	25.7 ± 0.4	2.6 ± 0.3	26.3 ± 1.8
5	2.5 ± 0.2	24.2 ± 1.8	2.5 ± 0.1	19.4 ± 1.7	2.6 ± 0.4	25.8 ± 2.0	2.3 ± 0.1	24.5 ± 0.4	2.5 ± 0.2	27.2 ± 3.1
26	2.6 ± 0.2	24.2 ± 0.8	2.7 ± 0.4	20.9 ± 0.4	2.6 ± 0.1	25.2 ± 0.9	2.6 ± 0.1	26.3 ± 1.2	2.6 ± 0.1	25.8 ± 0.6
60	2.6 ± 0.1	22.9 ± 0.4	2.6 ± 0.3	19.7 ± 0.0	2.8 ± 0.1	23.3 ± 0.8	2.6 ± 0.1	24.4 ± 0.3	-	-

[a]T: control wine with no added yeast lees; BE: wine with older fermented wine yeast lees from six-month wood aging; LN: wine with newly fermented yeast lees.

Table 3 Sensory results from a tasting panel on three wines (mean ± SD).

	T[a]	BE[a]	LN[a]
Color intensity	3.27 ± 0.79	3.27 ± 0.65	3.55 ± 0.69
Aroma			
Fruity	2.73 ± 0.79	2.09 ± 1.04	2.27 ± 1.01
Floral	1.82 ± 0.87	1.73 ± 1.01	1.73 ± 1.10
Intensity	2.82 ± 0.98	2.82 ± 0.60	2.73 ± 0.90
Persistency	2.55 ± 0.82	2.64 ± 0.67	2.36 ± 0.92
Equilibrium	2.55 ± 1.04	2.09 ± 0.54	2.45 ± 1.04
Mouthfeel			
Body	3.27 ± 0.47	2.64 ± 0.67	3.45 ± 0.52
Bitterness	2.27 ± 0.90	2.36 ± 0.81	2.36 ± 0.67
Astringency	2.82 ± 0.75	2.64 ± 0.67	2.73 ± 0.79
Acidity	3.00 ± 0.63	2.55 ± 0.82	3.09 ± 0.70
Persistency	3.09 ± 0.54	2.55 ± 0.52	2.73 ± 0.65
Equilibrium	2.82 ± 0.75	2.55 ± 0.52	3.18 ± 0.75
Overall balance	2.91 ± 0.94	2.36 ± 0.81	3.00 ± 0.89

[a]T: control wine with no added yeast lees; BE: wine with older fermented wine yeast lees from six-month wood aging; LN: wine with newly fermented yeast lees.

its initial concentration, with no saturation on the adsorption process. In the same way, if the amount of added yeast lees is higher, there will be a greater decrease in anthocyanin content. Mazauric and Salmon (2005) have shown that polyphenol adsorption by yeast lees occurs with two-step kinetics, starting with a rapid polyphenol fixing followed by a slow and constant saturation fixing, which is in accordance with the results obtained in this work. This decrease also occurs in the polymeric pigments initially.

For the evolution of hue, the fact that the BE wine had a higher value at the end of the experiment than the T and LN wines could be due to its higher level of added yeast lees, resulting in the preferential adsorption of the blue anthocyanins and maintaining more yellow compounds in the wine medium (Morata et al. 2003, Mazauric and Salmon 2006).

The evolution of the color parameters showed that the addition of external yeast lees to this red wine did not influence color stabilization during the studied period, as also reported elsewhere (Guadalupe et al. 2007, Guadalupe and Ayestarán 2008). The only major observation was the interaction between yeast lees and color compounds, resulting in a decrease of colored anthocyanins as reported elsewhere (Vasserot et al. 1997, Salmon et al. 2002, Morata et al. 2003, Mazauric and Salmon 2005, 2006). Color stability is mainly due to the presence of polymeric pigments, molecules resulting from the reaction between anthocyanins and tannins. The phenomena that enhance the formation of polymeric pigments or that avoid its precipitation result in a stabilization of color. Regarding the wine color during the studied period, the precipitation of polymeric pigments in any modality was not observed. On the other hand, it was not expected that the presence of polysaccharides could enhance the formation of polymeric pigments.

Yeast lees and proanthocyanidin evolution. There was an initial decrease in total proanthocyanidin content between day 0 and day 5 in all wines, a decrease more noticeable in wine with added yeast lees. This result can be due to the adsorption of condensed tannins by the lees, as suggested elsewhere (Mazauric and Salmon 2005, Fernández et al. 2011). After day 5, the decrease in proanthocyanidin content was faster in the T control wine, although it ended with a higher proanthocyanidin content. If the decreased rate observed in the first three months of this experiment was maintained throughout the aging time, then the addition of yeast lees would be expected to stabilize the decrease of proanthocyanidins. It has already been observed that some mannoproteins

can influence the aggregation and growth of tannin particles (Riou et al. 2002).

The proanthocyanidin distribution profile for the T wine showed that more polymerized proanthocyanidins precipitated throughout time. The absence of proanthocyanidins with an mDP between 20 and 33 on day 60 was probably due to three factors: (1) the polymerization of flavan-3-ol units results in the formation of high molecular weight polymers that precipitate; (2) the association between tannins and anthocyanins as $T\text{-}A^+$ complexes promotes the stability of the molecule without any further growth (Vidal et al. 2002); and (3) as tannin tends to aggregate through time and further to precipitate, a reduction of the number of flavan-3-ol units existing on solution occurs, limiting the reaction between monomers and monomers with oligomers in order to form higher-chain polymers.

In comparing the evolution of proanthocyanidins in BE wine and T wine, it appears that the addition of old yeast lees promoted an initial adsorption of the highly polymerized proanthocyanidins, followed by the maintenance of the low and medium molecular weight proanthocyanidins in the wine medium. This confirmed that the yeast lees have a stronger interaction with highly polymerized condensed tannins, as reported by Mazauric and Salmon (2006), although the highest mDP found by these authors was 16. Guadalupe and Ayestarán (2008) also verified that in a wine treated with commercial mannoproteins there was a decrease of high molecular weight mannoproteins at the same time that proanthocyanidins decreased, suggesting that high molecular weight mannoproteins aggregate to proanthocyanidins and that these coaggregates precipitate or flocculate. On the other hand, the maintenance of the low and medium molecular weight proanthocyanidins in the wine medium can be attributed to the low and medium molecular weight mannoproteins that were extracted from the yeast lees during the experiment period. Riou et al. (2002) verified that in a wine model solution, polysaccharides, namely mannoproteins, interfere with the particle size of proanthocyanidins, although they do not protect the tannins' initial aggregation.

In the LN wine, there was a higher percentage of proanthocyanidins with an mDP between 14 and 20 on the last day of the trial. That could be due to a retarding of higher molecular weight proanthocyanidin polymerization promoted by mannoproteins released by the added yeast lees.

Concerning the evolution of wine proanthocyanidin galloylation and prodelphinidin percentage, the decrease of prodelphinidin percentage is in accordance with other results (Mazauric and Salmon 2005), in which the condensed tannins that remained in the wine had fewer epigallocatechin units than the initial tannins, indicating that the more polar condensed tannins are preferentially adsorbed by yeast lees. The decrease of galloylation percentage was previously reported (Mazauric and Salmon 2006) and showed that the condensed tannins adsorbed by yeast lees had a high galloylation percentage when compared with the wine initial condensed tannins. In the current study, proanthocyanidins with a higher number of hydroxyl groups tended to disappear more rapidly from the BE wine. Cheynier et al. (1992) showed that the more galloylated proanthocyanidins were more rapidly involved in condensation reactions than the nongalloylated compounds.

In comparing the evolution of total proanthocyanidin content and the evolution of different proanthocyanidin mDP intervals and galloylation and prodelphinidin percentages, it appears that there were two different mechanisms acting as a result of the external yeast lees addition. The first was observable in the transition from day 0 to day 5, where the external lees addition promoted a rapid adsorption of the proanthocyanidins, namely the ones that were more polymerized, as seen in the results for the BE wine. Second, after this rapid adsorption, it seemed that there was a retarding of the proanthocyanidin polymerization to a higher degree, namely maintaining a relatively high amount of proanthocyanidins in the 8–14 mDP interval for the BE wine and in the 14–20 interval for the LN wine, probably due to the mannoproteins released by yeast lees. The first effect, related to yeast lees adsorption, was more evident in the BE wine, and it was the result of adding a higher amount of yeast lees to this wine than to the LN wine. The second effect, related to the retarding of further polymerization due to the presence of mannoproteins, was more evident in the LN wine. As the LN wine yeast lees were fresh, removed at the very end of alcoholic fermentation, they possibly had more effective mannoproteins available for the stabilization phenomenon than the ones from BE wine yeast lees. It should be also noticed that the amount of protein in the LN FR fraction is higher than in the BE FR fraction.In LN FR, peak 1 contained 74.1% proteins and peak 2 contained 18.2%. In BE FR, the same peaks contained 14.8% and 7.3%, respectively. The protein composition of these macromolecules may influence their interaction with proanthocyanidins. Poncet-Legrand et al. (2007) verified that low and medium molecular weight mannoproteins (51 and 62 kDa) prevented polyphenol precipitation by preventing tannin aggregation. Both yeast lees used had mannoprotein with an average molecular weight of 54 and 50 kDa, respectively; however, this mannoprotein was more perceptible in the polysaccharide profile of the LN wine with newly fermented yeast lees. The tannin polymerization retarding effect could have been promoted by the interaction of these particular mannoprotein with the proanthocyanidins in the wine medium.

Sensory analysis. The wine with newly fermented yeast lees (LN) received the highest ratings from the tasting panel. The wine with older fermented yeast lees (BE) had lower ratings in wine body, persistence, and mouthfeel equilibrium. These results were in accordance with results for proanthocyanidin content. The added yeast lees removed more condensed tannins from the medium, resulting in a wine with lower mouthfeel equilibrium. This way, even if the lees addition contributed to an enrichment of wine mannoproteins, they could not compensate, regarding the sensory characteristics, the high decrease in wine tannins. In BE wine, the amount of added yeast lees was enough to remove some tannins that could contribute to a more aggressive wine, softening the

mouthfeel. The mannoproteins that were extracted to this wine may also have contributed to improved mouthfeel.

Conclusions

The addition of external white wine yeast lees did not have an effect on wine color stabilization. The only remarkable effect was the decrease of anthocyanin and polymeric pigment content, particularly the colored anthocyanins, immediately after the addition of yeast lees. This decrease was due to the adsorptive interactions that were established between anthocyanins and yeast lees. The same kind of effect was observed between proanthocyanidins and yeast lees at the beginning of the experiment. During the time, after the initial decay, it appeared that there was a retarding effect on the proanthocyanidin polymerization reactions by the addition of external yeast lees. This effect could occur due to two factors: the rapid initial removal of the more polar proanthocyanidins by adsorption to yeast lees and the proanthocyanidin polymerization retarding effect promoted by the low and medium molecular weight mannoproteins extracted from the added yeast lees. Regarding proanthocyanidin structure, the addition of older fermented yeast lees obtained from a six-month aging process (BE wine) resulted in the prevalence of less polar tannins in wine and an adsorption of the highest molecular weight proanthocyanidins. The fact that BE wine was rated by the tasting panel as less equilibrated in mouthfeel could be due to the greater amount of external white wine yeast lees that was added to the wine. On the other hand, at the end of the experiment the LN wine, with newly fermented yeast lees, had a higher percentage of proanthocyanidins in the 14–20 mDP interval, and, possibly due to this fact, a higher mouthfeel rating. These results show that the addition of external yeast lees in the winery has to be planned by the tasting panel and both the working concentration and the yeast lees age should be tested in a small volume before adding the yeast lees to the wine in the industrial process.

Literature Cited

Albersheim, P., D.J. Nevis, P.D. English, and A. Karr. 1967. A method for the analysis of sugars in plant cell-wall polysaccharides by gas liquid chromatography. Carbohydr. Res. 5:340-345.

Cheynier, V., J. Rigaud, and J.M. Ricardo da Silva. 1992. Structure of procyanidin oligomers isolated from grape seeds in relation to some of their chemical properties. *In* Plant Polyphenols. R.W. Hemingway and P.E. Laks (eds.), pp. 281-294. Plenum Press, New York.

Dallas, C., J.M. Ricardo da Silva, and O. Laureano. 1996. Products formed in model wine solutions involving anthocyanins, procyanidin B_2, and acetaldehyde. J. Agric. Food Chem. 44:2402-2407.

Dubois, M., K.A. Gilles, J.K. Hamilton, P.A. Rebers, and S. Smith. 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. 28:350-356.

Escot, S., M. Feuillat, L. Dulau, and C. Charpentier. 2001. Release of polysaccharides by yeasts and the influence of released polysaccharides on color stability and wine astringency. Aust. J. Grape Wine Res. 7:153-159.

Fernández, O., O. Martínez, Z. Hernández, Z. Guadalupe, and B. Ayestarán. 2011. Effect of the presence of lysated lees on polysaccharides, color and main phenolic compounds of red wine during barrel ageing. Food Res. Int. 44:84-91.

Feuillat, M., and C. Charpentier. 1982. Autolysis of yeasts in Champagne. Am. J. Enol. Vitic. 33:6-13.

Feuillat, M., M. Freyssinet, and C. Charpentier. 1989. L'élevage sur lies des vins blancs de Bourgogne. II. Evolution des macromolécules: Polysaccharides et protéines. Vitis 28:161-176.

Fornairon-Bonnefond, C., C. Camarasa, M. Moutounet, and J.M. Salmon. 2002. New trends on yeast autolysis and wine ageing on lees: A bibliographic review. J. Int. Sci. Vigne Vin 36:49-69.

Fulcrand, H., S. Remy, J.M. Souquet, V. Cheynier, and M. Moutounet. 1999. Study of wine tannin oligomers by on-line liquid chromatography electrospray ionization mass spectrometry. J. Agric. Food Chem. 47:1023-1028.

Gonçalves, F., A. Heyraud, M.N. Pinho, and M. Rinaudo. 2002. Characterization of white wine mannoproteins. J. Agric. Food Chem. 50:6097-6101.

Guadalupe, Z., and B. Ayestarán. 2008. Effect of commercial mannoprotein addition on polysaccharide, polyphenolic, and color composition in red wines. J. Agric. Food Chem. 56:9022-9029.

Guadalupe, Z., A. Palacios, and B. Ayestarán. 2007. Maceration enzymes and mannoproteins: A possible strategy to increase colloidal stability and color extraction in red wines. J. Agric. Food Chem. 55:4854-4862.

Haslam, E. 1980. *In vino veritas*: Oligomeric procyanidins and the ageing of red wines. Phytochemistry 19:2577-2582.

Hernawan, T., and G. Fleet. 1995. Chemical and cytological changes during the autolysis of yeasts. J. Ind. Microbiol. Biotechnol. 14:440-450.

Labarbe, B., V. Cheynier, F. Brossaud, J.M. Souquet, and M. Moutounet. 1999. Quantitative fractionation of grape proanthocyanidins according to their degree of polymerization. J. Agric. Food Chem. 47:2719-2723.

Lowry, O.H., N.J. Roserbrough, A.L. Farr, and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265-275.

Mazauric, J.P., and J.M. Salmon. 2005. Interactions between yeast lees and wine polyphenols during simulation of wine aging: I. Analysis of remnant polyphenolic compounds in the resulting wines. J. Agric. Food Chem. 53:5647-5653.

Mazauric, J.P., and J.M. Salmon. 2006. Interactions between yeast lees and wine polyphenols during simulation of wine aging. II. Analysis of desorbed polyphenol compounds from yeast lees. J. Agric. Food Chem. 54:3876-3881.

Medina, K., E. Boido, E. Dellacassa, and F. Carrau. 2005. Yeast interactions with anthocyanins during red wine fermentation. Am. J. Enol. Vitic. 56:104-109.

Moine-Ledoux, V., A. Perrin, I. Paladin, and D. Dubourdieu. 1997. Premiers résultats de stabilisation tartrique des vins par addition de mannoprotéines purifiées (Mannostab). J. Int. Sci. Vigne Vin 31:23-31.

Monagas, M., C. Gómez-Cordovés, B. Bartolomé, O. Laureano, and J.M. Ricardo-da-Silva. 2003. Monomeric, oligomeric, and polymeric flavan-3-ol composition of wines and grapes from *Vitis vinifera* L. cv. Graciano, Tempranillo, and Cabernet Sauvignon. J. Agric. Food Chem. 51:6475-6481.

Morata, A., M.C. Gómez-Cordovés, J. Suberviola, B. Bartolomé, B. Colomo, and J.A. Suárez. 2003. Adsorption of anthocyanins by yeast cell walls during the fermentation of red wines. J. Agric. Food Chem. 51:4084-4088.

OIV. 2006. Recueil des Méthodes Internationales d'Analyse des Vins et Moûts. Organisation International de la Vigne et du Vin, Paris.

Poncet-Legrand, C., T. Doco, P. Williams, and A. Vernhet. 2007. Inhibition of grape seed tannin aggregation by wine mannoproteins: Effect of polysaccharide molecular weight. Am. J. Enol. Vitic. 58:87-91.

Ribéreau-Gayon, P., D. Dubourdieu, B. Donéche, and A. Lonvaud. 1998. La développement des bactéries lactiques dans le vin. *In* Traité

d'Œnologie. 1. Microbiologie du Vin: Vinifications, pp. 197-223. Dunod, Paris.

Ricardo da Silva, J.M., J.P. Rosec, M. Bourzeix, and N. Heredia. 1990. Separation and quantitative determination of grape and wine procyanidins by high performance reversed phase liquid chromatography. J. Sci. Food Agric. 53:85-92.

Rigaud, J., J. Perez-Ilzarbe, J.M. Ricardo da Silva, and V. Cheynier. 1991. Micro method for the identification of proanthocyanidin using thiolysis monitored by high-performance liquid chromatography. J. Chromatogr. 540:401-405.

Riou, V., A. Vernhet, T. Doco, and M. Moutounet. 2002. Aggregation of grape seed tannins in model wine: Effect of wine polysaccharides. Food Hydrocoll. 16:17-23.

Salmon, J.M., C. Fornairon-Bonnefond, and J.P. Mazauric. 2002. Interactions between wine lees and polyphenols: Influence on oxygen consumption capacity during simulation of wine aging. J. Food Sci. 67:1604-1609.

Somers, T.C. 1971. The polymeric nature of wine pigments. Phytochemistry 10:2175-2186.

Somers, T.C., and M.E. Evans. 1977. Spectral evaluation of young red wines: Anthocyanin equilibria, total phenolics, free and molecular SO_2, "chemical age." J. Sci. Food Agric. 28:279-287.

Sun, B., G.P. Belchior, J.M. Ricardo da Silva, and M.I. Spranger. 1999. Isolation and purification of dimeric and trimeric procyanidins from grape seeds. J. Chromatogr., A 841:115-121.

Vasserot, Y., S. Caillet, and A. Maujean. 1997. Study of anthocyanin adsorption by yeast lees. Effect of some physicochemical parameters. Am. J. Enol. Vitic. 48:433-437.

Vidal, S., D. Cartalade, J.M. Souquet, H. Fulcrand, and V. Cheynier. 2002. Changes in proanthocyanidin chain length in winelike model solutions. J. Agric. Food Chem. 50:2261-2266.